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Detection and molecular characterization of feline hemoplasmas in wild felid species in Iran in the Middle East.

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Short running title; Feline hemoplasmas in wild felid species in Iran

Abstract

Three feline hemoplasma species exist in felids: *Mycoplasma haemofelis,* '*Candidatus* Mycoplasma haemominutum', and '*Candidatus* Mycoplasma turicensis'.

The aims of the study were to determine the presence of, and molecularly characterize, any hemoplasmas in wild felids, including the endangered Persian leopard in Iran, the Middle East.

Blood samples were collected from 19 wild felids, including three Persian leopards. Using species-specific hemoplasma PCRs and ELISA serological testing for feline leukaemia virus and feline immunodeficiency virus (FIV), two Persian leopards were found to be infected with '*Ca.* M. haemominutum' and were seropositive for FIV. Partial 16S rRNA gene sequences were generated for these '*Ca.* M. haemominutum' species and subsequent phylogenetic analysis revealed 97.70% to 99.45% sequence identity with those found in domestic cats from Iran and other countries.

This study confirms the presence of '*Ca.* M. haemominutum' and concurrent FIV antibody in wild felids in Iran. This represents the first report of hemoplasma in wild felids in the Middle East as well as the first report of infection in Persian leopards.

Key words; Feline hemoplasma, *Panthera pardus saxicolor,* Persian leopard.

1. Introduction

Hemoplasmas are hemotropic mycoplasmal bacteria that infect a wide range of mammals [1, 2]. At least three feline hemoplasma species have been described in domestic cats including *Mycoplasma haemofelis,* '*Candidatus* Mycoplasma haemominutum', and '*Candidatus* Mycoplasma turicensis' [1-4]. The most pathogenic species is *M. haemofelis*, which can cause hemolytic anemia [5, 6] in immunocompetent cats. Coinfection of hemoplasmas with other pathogens such as feline leukemia virus (FeLV) and feline

immunodeficiency virus (FIV) may worsen the severity of the hemoplasma-induced anemia and result in anemia following infection with less pathogenic hemoplasmas such as '*Ca.* M. haemominutum', and '*Ca.* M. turicensis' [7, 8].

Hemoplasma infection with *M. haemofelis*, '*Ca.* M. haemominutum' and/or '*Ca.* M. turicensis' has been reported in around nine wild felid species worldwide [9-11], with wildlife isolates showing near identity to those found in domestic feline species [9]. There are, however, only limited studies of hemoplasma infections in wild felids, and no studies have yet been performed in countries in the Middle East, such as Iran, and the natural transmission route for hemoplasmas is not known [9].

The Persian leopard is an endangered wild felid, native to Iran and some neighboring countries. Following the extinction of the lion *Panthera leo persica* and tiger *Panthera tigiris virgate* in Iran, it is the only large wild felid now existing in Iran [12-15], and no studies have yet evaluated this species as a host for feline hemoplasma infection. We have recently reported the presence and molecular characterization of feline hemoplasma infections in domestic cats in Iran [16], and the aim of this study was to document the presence and molecularly characterize of feline hemoplasma species in wild felids in Iran in the Middle East.

2. Materials & Methods

2.1. Sample Collection and Processing

Nineteen EDTA-anticoagulated blood samples (FL Medical K3 EDTA K3E, Lot. F111332 2.5 mL tube, Torreglia, Italy) were obtained from the following cats; twelve African lions, four leopards (three Persian leopards and one African leopard), one Eurasian lynx, one Bengal tiger and one Caracal, using a blowpipe filled with a combination of drugs and dosage to each species; ketamine (3mg/kg) and medetomidine (0.03 mg/kg) for Bengal tiger, Persian and African leopards, butorphanol (0.2 mg/kg), medetomidine (0.035mg/kg) and midazolam

(0.15 mg/kg) for Eurasian lynx and Caracal caracal and tiletamine/zolazepam (1.5 mg/kg) and medetomidine (0.015 mg/kg) for African lion. These are given intramuscularly to anesthetize the animals followed by femoral vein sampling. Approval was granted for the study from the Iran Veterinary Organization since samples were taken as part of a national and international cooperative project for conservation of Persian leopards, supported by the Iranian Department of the Environment, the International Union for Conservation of Nature, The Wildlife Conservation Society and Panthera. The sampled animals were kept either in Tehran zoo or in the Tandoureh National Park. Tandoureh National Park has been protected since 1968 and is located in north eastern Iran and is around 355 km^2 in size. Signalment data for these wild felids, as well as their origin and current residence are shown in Table 1.

Hematological parameters including white blood cell, red blood cell (RBC), Hematocrit (HCT), hemoglobin concentration (Hb), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelets were measured using an automatic hemocytometer (Hema-screen 18, Hospitex diagnostic, Florence, Italy). Blood smears were prepared for differential white blood cells count and examination for hemoparasites. Plasma was submitted for serological retrovirus testing for FeLV and FIV using a commercially available rapid diagnostic ELISA kit (Quicking FIV Ab + FeLV Ag Combined Test, W81099, China), according to the manufacturer's instructions, and were confirmed by repeat ELISA testing using a different serological retrovirus test (ELISA kit for serodiagnosis of FeLV and FIV Ab, Biopronix, Agrolabo, Italy).

2.2. DNA Extraction

DNA was extracted from 100 µl whole blood from each sample using a commercial kit (QIAamp cador pathogen Mini kit, Qiagen, Hilden, Germany), following the manufacturer's instructions, and stored at -20℃ until further use.

Distilled water and known positive blood samples for each of the three feline

hemoplasma species, obtained from the School of Veterinary Sciences, University of Bristol, Bristol, UK and Bologna University, Bologna, Italy, were used as negative and positive controls respectively during each run of DNA extractions.

2.3. Diagnostic Polymerase Chain Reaction (PCR) assays

A control conventional PCR to amplify a fragment of feline glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was performed to detect possible PCR inhibitors in DNA samples [17]. Screening hemoplasma PCR analysis was performed using a previously described generic universal hemoplasma conventional PCR assay using 5′-ATACGGCCCATATTCCTACG-3′ and 5′-TGCTCCACCACTTGTTCA-3′ as forward and reverse primers, respectively [18].

All samples were then subjected to species-specific conventional PCRs for each of the three feline hemoplasma species using previously described conventional PCR assays [19, 20]. Positive controls of *M. haemofelis*, '*Ca.* M. haemominutum' and '*Ca.* M. turicensis' were used for both the generic haemoplasma and species specific PCRs.

2.4. 16S rRNA Gene Sequencing

The 16S rRNA gene of positive samples on generic screening hemoplasma PCR was amplified using primers 8F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-GGTTACCTTGTTACGACTT-3′, as previously described, with resulting PCR products then subjected to sequencing using the Sanger technique (ABI, 96-capillary XL) [21]. After evaluating the quality of sequence reading in Finch TV software (Geospiza), 5' and 3' ends of the forward and reverse sequence reading were trimmed. The forward and reverse sequences of each sample were then overlapped and aligned with available 16S rRNA sequences of '*Ca.* M. haemominutum' in Genbank. Finally, partial 16S rRNA sequences of 1086bp (lacking about 200 bp from each 5' and 3' end of complete 16S rRNA sequence of '*Ca.* M. haemominutum') were obtained.

2.5. Statistical Analysis

Data analysis, including descriptive statistics, was performed using SPSS software (16.0 IBM, New York, USA). African lion hematology reference intervals were calculated using the mean ± SD data available in previously published work [22], using the formula mean±1.96SD. Sequence data analysis and phylogenic tree construction were performed with MEGA6 software using the partial 16S rRNA sequences derived in this study as well as other wild and domestic cat hemoplasma sequences downloaded from Genbank (Accession numbers shown in Figure 1). Bootstrap testing (1000 replicates) and out-grouping were used to validate the phylogenic tree [23]. The evolutionary distances were computed using the Kimura 2-parameter method [24] and the Neighbor-Joining method [25] used for tree construction [26].

Nucleotide Sequence Accession Numbers. The partial 16S rRNA gene sequences derived from this study were submitted to Genbank with accession numbers KU852586 and KU852587.

3. Results

Of the 19 samples analyzed, all were PCR-positive for GAPDH, and two (10.5%) were PCR-positive by generic universal hemoplasma conventional PCR. Only the same two samples were positive on species-specific PCR; both for '*Ca.* M. haemominutum' only. All positive controls had expected amplified band in the generic universal and the species-specific conventional haemoplasma PCRs and distilled water as the negative control had none. Both of the positive samples were from old (14 and 15 years) male Persian leopards (Case numbers 13 and 14 in Table 1), from two different geographical areas of Iran. No hemoplasma organisms were observed on blood smear examination.

The two hemoplasma ('*Ca.* M. haemominutum') infected Persian leopards were both also FIV seropositive, and one African lion was also FeLV and FIV positive but not

hemoplasma infected. No other samples were retrovirus positive.

To the authors' knowledge, no hematological reference ranges exist for Persian leopards, nor for any closely related species (e.g. African leopard, Arabian leopard), limiting interpretation of the hematology profiles of the Persian leopards in the current study. However, as shown in Table 2, the hematology profiles of the two '*Ca.* M. haemominutum' and FIV-seropositive Persian leopards (Case numbers 13 & 14) showed HCT, Hb, and RBC counts at the lower end of the reference range used for domestic cats, and HCT and RBC counts below the reference range calculated for African lions based on Larsson et. al 2015 [22], and were lower than those recorded in the non-infected Persian leopard (Case number 15). Thus it is possible that '*Ca.* M. haemominutum' and FIV infection were associated with a reduction in erythrocyte indices in the infected cats, but further data from larger numbers of cats would be required to confirm this.

The hematology profiles of the 12 African lions were also compared to the reference range calculated for African lions based on Larsson et. al 2015 [22], and 11 of the 12 lions had HCT and Hb values within or above the reference range. The FeLV and FIV seropositive but hemoplasma PCR negative lion had a hypochromic normocytic anemia (HCT 20.7%). The four remaining cases (Case numbers 16, 17, 18 and 19) could not have their hematological profiles determined due to sample hemolysis.

The partial (1086 bp) '*Ca.* M. haemominutum' 16S rRNA gene sequences derived for the two hemoplasma infected Persian leopards in the current study (KU852586 and KU852587) showed high sequence identity (97.7-99.45%) with, and were closely related to the '*Ca.* M. haemominutum' sequences in Genbank derived from worldwide wild felids and domestic cats [9, 27-29], including Iranian domestic cats. Data are shown in Figure 1. The '*Ca.* M. haemominutum' Persian leopard sequence KU852586 was slightly more closely related to the Iranian domestic cat sequence KU852585 than the other Persian leopard

sequence KU852587, with 99.26% sequence identity. The sequence identity between the two Persian leopards '*Ca.* M. haemominutum' (KU852586 and KU852587) was 98.43%. The highest sequence identities of 99.63% and 98.62% were between '*Ca.* M. haemominutum' sequences KU852586 and KU852587 derived from Persian leopards and DQ825452 from a lion in Tanzania.

4. Discussion

This study documents the presence of hemoplasmas in wild felids for the first time in Iran in the Middle East. It is also the first documentation of hemoplasma infection in the endangered Persian leopard species [12]. The prevalence of hemoplasma infection in wild felids has varied in different studies but is not frequently high. In a surveillance study in Brazil on neotropic and exotic felids, 9.2% of 109 felids were hemoplasma positive (all '*Ca.* M. haemominutum') [30], whilst in free-ranging Cheetahs in Namibia, only one of 63 Cheetahs was positive [31]. However higher prevalence was reported in another study evaluating a large sample size (275) from worldwide geographical areas where prevalence of 18%, 32% and 20% were found for *M. haemofelis,* '*Ca.* M. haemominutum', and '*Ca.* M. turicensis', respectively [9]. In the current study, hemoplasma infection was confirmed in just two of 19 samples (10.5%), although the sample size was small since access to wild felid samples in the Middle East is very limited due to the difficulties in access to hosts and collection of blood. Both '*Ca.* M. haemominutum' infected wild felids in the current study were old male Persian leopards, an indigenous species in Iran. This is in agreement with other studies in domestic cats showing that being male and, older, are risk factors for '*Ca.* M. haemominutum' infection [27, 32-37]. Fighting behavior is also regarded as a risk factor for hemoplasma infection [27, 36, 38], although the fighting behavior of the cats sampled was not completely known and leopards (*Panthera pardus*) are generally not regarded as an aggressive species [39]. However, in the literature there are cases of intraspecific killing

among leopards over a kill, territory or cannibalism, so aggression is possible [40, 41]. There are also two reports of intraspecific killing from Persian leopards in Tandoureh in 2007 and 2016 over food and territory (Memarian. I, personal communication).

Neither of the two '*Ca.* M. haemominutum' infected Persian leopards identified in the current study lived in zoos. As reported in an extensive study on feline hemoplasma infection in wild felid species worldwide[9], free-ranging felids had higher hemoplasma infection prevalence [9, 42, 43] than captive felids. This may be because free-ranging felids have more fighting and hunting habits and/or more exposure to vectors, than captive or zoo-based felids. In the same study described, a significant correlation between FeLV PCR positivity and hemoplasma infection was found in European wild cats[9]. There are several reports of retrovirus infections in free-ranging and captive wild felids [44-47], and multiple other concurrent infections such as feline calicivirus, feline herpesvirus, feline parvovirus, and feline coronavirus [42, 43]. In the current study both '*Ca.* M. haemominutum' Persian leopards were FIV seropositive and this is, to the authors' knowledge, the first report for such a co-infection in a wild felid species.

It is not known if the '*Ca.* M. haemominutum' infection in the Persian leopards caused anemia. This was difficult to assess since no reference ranges exist for hematological parameters in this species, and it is known that greater anemia can occur in cats with concurrent '*Ca.* M. haemominutum' and retrovirus infection compared to '*Ca.* M. haemominutum' alone [7]. The very small sample size did not permit a statistical comparison between '*Ca.* M. haemominutum' FIV-seropositive and FIV-seronegative Persian leopards. Nevertheless, it was of note that the HCT, Hb and RBC counts of the two '*Ca.* M. haemominutum' FIV-seropositiveleopards were lower than the Persian leopard free from hemoplasmas and retroviral infection, suggesting that coinfection of '*Ca.* M. haemominutum' and FIV could have been associated with reduced RBC parameters.

The partial 16S rRNA gene phylogenetic analysis found that the '*Ca.* M. haemominutum' isolates derived from this study were closely related to those from different geographical origins and from both domestic and wild felids. In a previous phylogenetic study of domestic feline hemoplasmas, using both 16S rRNA gene and RNaseP genes, almost 100% identity was reported between Europe, Asia, Africa and United States species [48]. In a Japanese study, the identities of the detected hemoplasma sequences was very high, such that it was not possible to assume the origin of *M. haemofelis* and '*Ca.* M. turicensis' from endangered Iriomote cats. In agreement with our findings, previous studies describing feline hemoplasma phylogenetic analysis based on the RNaseP gene revealed similar close relationships between the hemoplasma species of both domestic and wild felids [9, 31, 48].

A limitation of this study is the small sample size, but despite this, it is interesting to note that two of the three Persian leopards tested were '*Ca.* M. haemominutum' positive, suggesting that hemoplasma infection may be prevalent in this species, especially as the two positive Persian leopards were from geographically distinct areas.

In conclusion, we have documented that hemoplasma infections occur in wild felids and we have reported, for the first time, hemoplasma infection in wild felids in the Middle East and hemoplasma infection in Persian leopards. Interestingly the two '*Ca.* M. haemominutum' infected Persian leopards were seropositive for FIV. The prevalence of infectious diseases in wild felids is difficult to assess and monitor but should be considered by those working to save endangered animal species such as the Persian leopard.

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Figure. 1. Phylogenetic analysis of partial 16S rRNA gene sequences from "*Candidatus* Mycoplasma haemominutum" isolates from Persian leopards. Sequences from this study are shown all in bold. Bootstrap values are given at the nodes of the tree. The following sequences are shown: *Mycoplasma haemofelis* (Cat, South Africa AF548631; Cat, Iran, KU852584; Eurasian Lynx, Switzerland, DQ825458; European Wildcat, France, DQ825441; *Leopardus Weidii*, Brazil, DQ825438; Cat, Switzerland, DQ157160; Cat, United Kingdom, AY150985; Cat, Australia, AY150977; Cat, Australia, AY150976; Cat, France, AY150972; Iberian Lynx, Spain, DQ825447; Cat, Iran, KU852583; Cat, United States, U95297, Lion, Tanzania, DQ825451), *Mycoplasma haemocanis* (Dog, United States, AF407208; Dog Germany AY150973), "*Candidatus* Mycoplasma haemomuris" (*Apodemus argenteus*, Japan, AB758437; wild mouse, Japan, U82963; *Apodemus argentus*, Japan, AB758436; Rattus rattus, Japan, AB758439) "*Candidatus* Mycoplasma turicensis" (Lion, Tanzania, DQ825454; *Leopardus Pardalis*, Brazil, DQ825448; Cat Switzerland, DQ157150; European Wildcat, France, DQ825450; *Mycoplasma haemolamae* AF306346, "*Candidatus* Mycoplasma haematoparvum" (Dog, United States, AY383241; Dog, United Kingdom, AY532390) "*Candidatus* Mycoplasma haemominutum" (Leopardus tigrinus, Brazil, DQ825439; Iberian Lynx, Spain, DQ825445; Cat, Israel, AY150974; Leopardus Weidii, Brazil, DQ825440; Ibnen Lynx, Spain, DQ825446; Ibnen Lynx, Spain, DQ825444; Euroasian Lynx, Switzerland, DQ825456; Persian Leopard, Iran, KU852587; Persian Leopard, Iran, KU852586; Lion, Tanzania, DQ825452; Cat, United Kingdom, AY150980; Cat United States, U88564; European Wildcat, France, DQ852442; Cat, United Kingdom, AY150981; Cat, Iran, KU852585), *Clostridium perfringens* NR 121697.

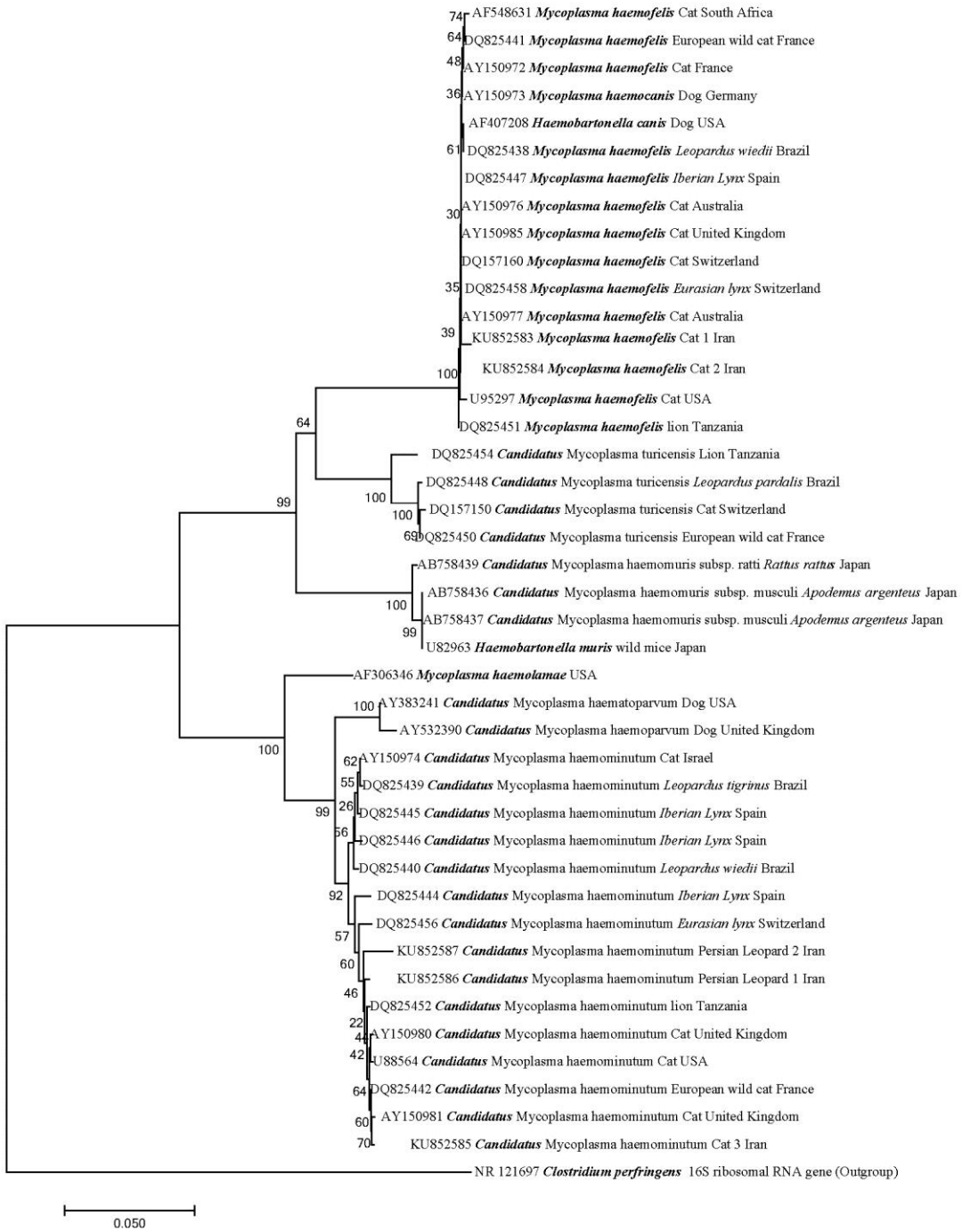
AF548631 Mycoplasma haemofelis Cat South Africa
DQ825441 Mycoplasma haemofelis European wild cat France
AY150972 Mycoplasma haemofelis Cat France
AY150973 Mycoplasma haemocanis Dog Germany
AF407208 Haemobartonella canis Dog USA
DQ825438 Mycoplasma haemofelis Leopardus wiedii Brazil
DQ825447 Mycoplasma haemofelis Iberian Lynx Spain
AY150976 Mycoplasma haemofelis Cat Australia
AY150985 Mycoplasma haemofelis Cat United Kingdom
DQ157160 Mycoplasma haemofelis Cat Switzerland
DQ825458 Mycoplasma haemofelis Eurasian lynx Switzerland
AY150977 Mycoplasma haemofelis Cat Australia
KU852583 Mycoplasma haemofelis Cat 1 Iran
KU852584 Mycoplasma haemofelis Cat 2 Iran
U95297 Mycoplasma haemofelis Cat USA
DQ825451 Mycoplasma haemofelis lion Tanzania
DQ825454 Candidatus Mycoplasma turicensis Lion Tanzania
DQ825448 Candidatus Mycoplasma turicensis Leopardus pardalis Brazil
DQ157150 Candidatus Mycoplasma turicensis Cat Switzerland
DQ825450 Candidatus Mycoplasma turicensis European wild cat France
AB758439 Candidatus Mycoplasma haemomuris subsp. ratti Rattus rattus Japan
AB758436 Candidatus Mycoplasma haemomuris subsp. musculi Apodemus argenteus Japan
AB758437 Candidatus Mycoplasma haemomuris subsp. musculi Apodemus argenteus Japan
U82963 Haemobartonella muris wild mice Japan
AF306346 Mycoplasma haemolamae USA
AY383241 Candidatus Mycoplasma haematoparvum Dog USA
AY532390 Candidatus Mycoplasma haemoparvum Dog United Kingdom
AY150974 Candidatus Mycoplasma haemominutum Cat Israel
DQ825439 Candidatus Mycoplasma haemominutum Leopardus tigrinus Brazil
DQ825445 Candidatus Mycoplasma haemominutum Iberian Lynx Spain
DQ825446 Candidatus Mycoplasma haemominutum Iberian Lynx Spain
DQ825440 Candidatus Mycoplasma haemominutum Leopardus wiedii Brazil
DQ825444 Candidatus Mycoplasma haemominutum Iberian Lynx Spain
DQ825456 Candidatus Mycoplasma haemominutum Eurasian lynx Switzerland
KU852587 Candidatus Mycoplasma haemominutum Persian Leopard 2 Iran
KU852586 Candidatus Mycoplasma haemominutum Persian Leopard 1 Iran
DQ825452 Candidatus Mycoplasma haemominutum lion Tanzania
AY150980 Candidatus Mycoplasma haemominutum Cat United Kingdom
U88564 Candidatus Mycoplasma haemominutum Cat USA
DQ825442 Candidatus Mycoplasma haemominutum European wild cat France
AY150981 Candidatus Mycoplasma haemominutum Cat United Kingdom
KU852585 Candidatus Mycoplasma haemominutum Cat 3 Iran
NR 121697 Clostridium perfringens 16S ribosomal RNA gene (Outgroup)
0.050

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Table 1. Signalment data, origin and residence of sampled wild felids

No.	Species	Scientific name	Gender	Age (years)	Origin	Residence at time of sampling
1	African lion	*Panthera leo*	Female	6	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
2	African lion	Panthera leo	Male	3	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
3	African lion	Panthera leo	Female	7	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
4	African lion	Panthera leo	Female	1	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
5	African lion	Panthera leo	Female	1	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
6	African lion	Panthera leo	Female	7	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
7	African lion	Panthera leo	Female	2	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
8	African lion	Panthera leo	Male	3	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
9	African lion	Panthera leo	Male	1	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
10	African lion	Panthera leo	Male	2	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
11	African lion	Panthera leo	Female	5	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
12	African lion	Panthera leo	Female	3	Born in zoos in Iran	Zoo of Pardisan, Tehran, Iran
13	Persian leopard	*Panthera pardus* ssp. *saxicolor*	Male	15	Born in wild in Khorasan, Iran	In wild in Iran (transferred to National Park of Tandooreh, Iran at time of sampling)
14	Persian leopard	Panthera pardus ssp. saxicolor	Male	14	Born in wild in Mazandaran, Iran before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
15	Persian leopard	Panthera pardus ssp. saxicolor	Female	4	Born in wild in Golestan, Iran before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
16	African leopard	*Panthera pardus* ssp. *pardus*	Male	22	Born in wild in Kenya before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
17	Eurasian lynx	*Lynx lynx*	Male	8	Born in wild in Iran before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
18	Caracal	*Caracal caracal*	Male	6	Born in wild in Iran before being transferred to National Park of Tandooreh, Iran	National Park of Tandoureh, Iran
19	Bengal tiger	*Panthera tigris* ssp. *Tigris*	Male	1.5	Born in zoo in Denmark	Zoo of Pardisan, Tehran, Iran

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Table 2. Hematological parameters for Persian leopards

Species	Persian leopard Case no. 13*	Persian leopard Case no. 14*	Persian leopard Case no. 15	Domestic cat Reference range [49]	African lion Reference range [22](mean ± SD)	Derived African lion Reference range**	Unit
Hct+	29,4	28,2	34,15	29-45	42,38 ± 4,73	33,11-51,65	%
Hb	10,2	8,8	12,1	8-14	14,11 ± 1,63	10,92-17,30	g/dl
RBC	6,1	6,59	7,92	6-10	8,97 ± 1,43	6,17-11,77	10^6/µl
MCV	48	50	48,5	41.0-54	47,70 ± 4,53	38,82-56,58	fl
MCH	12,8	15,7	14,53	13.3-17.5	15,48 ± 1,25	13,03-17,93	pg
MCHC	26,6	31	29,38	31-36	33,3 ± 2,02	29,34-37,26	%
Plt	139	102	98,5	2.3-6.8	-	-	10^5/µl
WBC	9,53	4,06	10,75	5.5-19.5	9,73 ± 1,43	6,93-12,53	10^3/µl
Seg.	7,1475	2,436	7,821	2.5-12.5	7,748 ± 1,209	5,38-10,12	10^3/µl
Band	0,1906	0,0406	0,131	0-0.3	0	0,00-0,00	10^3/µl
Lymph	1,906	1,421	2,281	1,5-7	894 ± 456	0,24-1787,76	10^3/µl
Mono	0,0953	0,0406	0,067	0-0.85	365 ± 193	0-743,28	10^3/µl
Eos	0,1906	0,0812	0,101	0-1.5	372 ± 364	0-1085,44	10^3/µl
Baso	0	0	0	Rare	0	0,00-0,00	10^3/µl

NB. No hematology reference range is available for Persian leopards.

* '*Ca.* M. haemominutum' FIV infected Persian leopards

+Hematocrit (HCT), hemoglobin concentration (Hb), Red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC), platelets (Plt), white blood cell count (WBC), segmented neutrophil (seg), band cell (Band), lymphocyte (Lymph), monocyte (Mono), eosinophil (Eos), basophil (Baso).

** Reference range for African lion derived using mean ± 1.96SD from Larrson et al [23]

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